

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/040972 A2

(51) International Patent Classification⁷: **A01N**

(21) International Application Number:
PCT/US2003/033140

(22) International Filing Date: 17 October 2003 (17.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/423,547 4 November 2002 (04.11.2002) US

(71) Applicant: **BIOSPHERE MEDICAL** [US/US]; 1050
Hingham Street, Rockland, MA 02370-1052 (US).

(72) Inventor: **KROM, James, A.**; 41 Trowbridge Stret, Bel-
mont, MA 02478 (US).

(74) Agents: **GORDON, Dana, M.** et al.; Patent Group, Foley
Hoag LLP, 155 Seaport Boulevard, Boston, MA 02210-
2600 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RADIOISOTOPE-ASSOCIATED POLYMERIC HYDROGEL MICROSPHERES AND METHODS FOR PRODUCING AND USING THE SAME

(57) Abstract: The present invention relates to polymeric materials that are labeled with colloidal metals, preferably colloidal gold, to processes for producing the labeled polymeric material, and to methods of using the materials in prophylactic, therapeutic and cosmetic applications. Specifically, the invention relates to porous injectable and implantable microparticles, preferably microspheres, that are associated with radioactive colloidal metals such that the microparticles are visible or detectable under regular light, by radiological and/or magnetic resonance imaging techniques, or both. The microparticles having radioactive colloidal metals are particularly useful for embolization, radiation therapy, drug delivery, gene therapy, and other prophylactic or therapeutic medical applications.



WO 2004/040972 A2

**RADIOISOTOPE-ASSOCIATED POLYMERIC
HYDROGEL MICROSPHERES AND METHODS
FOR PRODUCING AND USING THE SAME**

5

FIELD

The present disclosure generally relates to microspheres. More particularly, the present disclosure relates to polymeric hydrogels associated with radioactive metals, metal oxides, and the like.

10

BACKGROUNDColloidal Metals As Markers In Immunocytochemistry

Colloidal metals, especially colloidal gold, have a long history as staining agents in various applications. In 1857, Faraday speculated that the red color of colloidal gold
15 resulted from the reflection of the light, a property which became the basis for its initial use in light microscopy. Faraday, Philos. Trans. R. Soc. Lond. B. Biol. Sci., 147:145 (1857). Thiessen proved the particulate nature of colloidal gold in 1942. Thiessen et al., Kolloid Z., 101:241 (1942) Perhaps the first true applications in cell biology were by Harford et al., J. Biophys. Biochem. Cytol., 3:749 (1957) and Feldherr et al., J. Biophys. Biochem. Cytol.,
20 12:640 (1962), who used stabilized colloidal gold as an electron-dense tracer in cellular uptake and micro-injection experiments, respectively.

Since the publication by Faulk and Taylor in 1971 of "*An Immunocolloid Method For The Electron Microscope*," Faulk et al., Immunochemistry, 8:1081 (1971), colloidal metals, especially colloidal gold, have become a very widely used marker in light and
25 electronic microscopy. For example, colloidal gold has been used to detect a wide variety of cellular and extracellular constituents by *in situ* hybridization, immunogold, lectin-gold, and enzyme-gold labeling. Besides its use in light microscopic immunogold and lectin-gold silver staining, colloidal gold remains the label of choice for transmission electron microscopy studying thin sections, freeze-etch, and surface replicas, as well as for
30 scanning electron microscopy. However, the use of colloidal metal, especially colloidal gold, *in vivo*, has not been reported. Furthermore, using colloidal metals to label or staining a synthetic polymeric material has not been reported either.

Labeling of embolization materials

Therapeutic vascular embolization procedures are used to treat or prevent certain pathological situations *in vivo*. Most generally they are made using catheters or syringes under imaging control to position solid or liquid embolic agents in the target vessel.

5 Embolization can be used to occlude vessels of a variety of organs including brain, liver, and spinal cord, which results in reduced blood flow or complete occlusion of the vessels. One application of embolization is to stop or reduce blood flow in hemorrhagic situations. Another application is to stop delivery of vital blood supply and nutrients to tissue, for instance, to reduce or deny blood supply to a solid tumor. In the case of vascular
10 malformations, embolization enables the blood flow to the normal tissue, aids in surgery and limits the risks of hemorrhage. Depending on the pathological conditions, embolization can be used for temporary as well as permanent objectives.

 Embolization has been performed with a variety of materials such as small pieces of durable matters, including polyvinyl-alcohol irregular particles, liquid embolic products and
15 more recently with spherical-shaped solid hydrogels. A wide variety of commercially available embolic materials are difficult to see or to trace because they are relatively transparent, cannot be seen clearly with normal light before and during administration, or are difficult to detect after administration because they are not radiopaque and lack features that render them detectable on magnetic resonance imaging, ultrasound, or nuclear
20 medicine procedures.

 U.S. Patent Nos. 5,635,215 and 5,648,100 disclose an injectable microspheres comprising a hydrophilic acrylic copolymer coated with a cell adhesion promoter and a marking agent. Marking agents described in these patents include chemical dyes, magnetic resonance imaging agents, and contrast agents such as barium or iodine salts. Organic dyes
25 are complex molecules composed of aromatic structures and strong ionic charges. They are known especially in affinity chromatography as ligands for several biological structures. Their major limitation as markers for embolic agents are the possible dye release as a result of the hydrolysis of the dye-embolic material link with subsequent delivery in the blood stream. Another limitation of chemical dyes is that they may be absorbed to certain
30 biological structures or tissue, which may produce undesirable results. For example, it is well known in affinity chromatography that human albumin interacts strongly in physiological conditions with a dye named Cibacron Blue F3GA.

Thanoo et al. reported, in 1991, the preparation and properties of barium sulphate and methyl iothalamate loaded poly(vinyl alcohol) (PVA) microspheres as radiopaque particulate emboli. Thanoo, et al., Journal of Applied Biomaterials, 2:67 (1991). The barium sulphate and methyl iothalamate impregnated PVA microspheres reported therein
5 were prepared by the glutaraldehyde cross-linking of an aqueous dispersion of PVA containing the radiopaques in paraffin oil using dioctyl sulfosuccinate as the stabilizing agent and thionyl chloride as the catalyst.

Horák et al., in 1998, reported radiopaque poly(2-hydroxyethyl methacrylate) (HEMA) particles containing silver iodide complexes, which were tested on cell culture.
10 Horák et al., Biomaterials, 19:1303 (1998). The incorporation of silver iodide complexes inside the poly(HEMA) particles was achieved by first swelling the particles in potassium iodide solution and precipitating the silver iodide complexes using a 30 wt% solution of silver nitrate.

Although the methods mentioned above are efficient for staining of soft embolic
15 spherical agents, such as Embosphere® (a registered trademark of Biosphere Medical Inc.) or PVA microspheres, they may change the physical properties, such as density and compressibility, of the microspheres. Further, they may not provide good visibility, under regular light by naked eyes, for the particles before and during administration. The use of a coloring agent, such as chemical dye, is another possibility to stain the microspheres. But
20 the risk of this method is the release of dye molecules from the microspheres *in vivo*, as discussed above.

SUMMARY

The present disclosure provides polymeric materials that are associated with
25 radioactive particles. The radioactive particles can include radioactive metals, radioactive inorganic metal compounds, and other radioactive elements. The radioactive particles can include a beta-emitting radionuclide. A beta-emitting radionuclide can preferably also be a gamma-emitting radionuclide. A radionuclide can have a half life in a range of about 2 hours to about 30 days, preferably about 6 hours to about 120 hours. The radionuclides can
30 be derived from stable isotopes that have been radioactivated by neutron bombardment. This disclosure also provides processes for producing the labeled polymeric materials, injectable solutions and kits comprising the materials, and methods of using the materials in prophylactic and therapeutic applications. In one embodiment, the invention encompasses

polymeric materials containing radioactive particles (e.g., of metals or inorganic compounds of metals), preferably those of phosphorous, yttrium, iodine, gold, rhenium, or holmium. Preferred isotopes of iodine include ^{123}I , ^{125}I and ^{131}I . The materials can be hydrogels, substantially spherical, and may retain functions and properties of the original
5 polymeric materials. The materials are preferably detectable by radio-imaging techniques, such as by gamma camera.

In one aspect, the present invention is directed to a polymeric material associated with radioactive particles, e.g., comprising metals or inorganic metal compounds. Preferably, the polymeric material is a porous hydrogel. The radioactive particles can be
10 associated with the material within the pores. The material preferably may be detectable by radiological imaging techniques, for example, but not limited to, detection by gamma camera. The materials are further preferably implantable or injectable in humans or animals and are biocompatible and stable, with very little or no release of the radioactive particles within the body. Such metal containing polymeric materials can either form part
15 of a traditional prosthetic device or part of microparticles that are implantable or injectable for, embolization, or radiation therapy purposes.

In a preferred embodiment, the polymeric material is a hydrogel and comprises at least some of the radioactive particles within the pores therein. The polymeric material is preferably selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones,
20 styrenics, and polysaccharides. In another preferred embodiment, the polymeric material is implantable into a human.

The present invention also provides a microparticle which comprises a polymeric material associated with radioactive particles, e.g., of metal or metal-containing
25 compounds, wherein the microparticle is suitable for injection or implantation into a human.

In a preferred embodiment of the present invention, the microparticle comprises polymeric hydrogel material selected from one or more of the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide,
30 polyolefins, polyurethanes, silicones, styrenics, and polysaccharides. In another preferred embodiment, the polymeric material is porous. Further, the porous polymeric material may comprise at least part of the colloidal metal particles within the pores therein.

According to the present invention, the microparticle preferably comprises polymeric material that is an elastomer, a hydrogel, a water swellable polymer, or combinations thereof. More preferably, the polymeric material is an acrylic polymer, such as a trisacryl based acrylic polymer. In a most preferred embodiment, the material
5 comprises a hydrophilic acrylic copolymer that contains, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge.

Further, the neutral hydrophilic acrylic monomer is preferably selected from the
10 group consisting of acrylamides, methacrylamides and hydroxymethylmethacrylate; the difunctional monomer is preferably selected from the group consisting of N,N'-methylenebisacrylamide, N',N'-diallyltartradiamide, and glyoxal-bis-acrylamide; and the monomer having a cationic charge is preferably a monomer having a tertiary and/or quaternary amine function.

15 The microparticle of the present invention may further preferably comprises one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

The polymeric material, especially the microparticle, of the present invention may
20 optionally comprise traditional marking agents, such as a chemical dye, a magnetic resonance imaging agent, and/or a contrasting agent.

In yet another preferred embodiment of the present invention, the polymeric material is a poly(vinyl alcohol) ("PVA"), preferably a cross-linked PVA. The polymeric material of the present invention may also be a polymethacrylate, such as poly(methyl
25 methacrylate) or poly (2-hydroxyethyl methacrylate).

In another embodiment of the present invention, the polymeric material is in microparticle form with dimensions ranging from about 1 μm to about 2000 μm . In a preferred embodiment, the microparticles are substantially spherical microspheres with diameters ranging from about 10 μm to about 2000 μm , more preferably, from about 40 μm
30 to about 1200 μm . The microparticle of the present invention is preferably suitable for radiation therapy and/or therapeutic vascular embolization purposes.

The polymeric material of the present invention may contain pores both on the surface and within the body. Preferably, the pores have sizes, measured by the dimensions

of the cross sections, ranging from about 1 nm to about 10 μm , more preferably, from about 1 nm to about 1000 nm.

The radioactive particles contained within the polymeric material have dimensions ranging from about 1 nm to about 1000 nm and, preferably, from about 1 nm to about 500 nm. The radioactive particles are preferably selected from the group consisting of phosphorus, yttrium, iodine, gold, rhenium, and holmium. The radioactive particle can be a metal selected from the group consisting of gold, antimony, lanthanum, samarium, europium, terbium, holmium, ytterbium, lutetium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, scandium, platinum, palladium, copper, titanium, and chromium. Preferably, the radioactive particles are phosphorus, yttrium or iodine. Preferred isotopes of iodine include ^{123}I , ^{125}I and ^{131}I . Most preferably, the metal is gold.

In another preferred embodiment, the present invention provides a substantially spherical microparticle, or a microsphere, which comprises a hydrogel associated with radioactive colloidal gold particles, wherein the microsphere is suitable for injection or implantation into a human. In a more preferred embodiment, the present invention provides a microsphere having a diameter ranging between about 10 μm and about 2000 μm , useful for embolization, which comprises a hydrophilic acrylic copolymer associated with radioactive colloidal gold particles, wherein the hydrophilic acrylic copolymer comprises, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge. In an embodiment, a hydrogel can include at least 50% water by weight.

The microsphere of the present invention may also comprise one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents. Further, the microsphere may optionally comprise a marking agent selected from the group consisting of dyes, imaging agents, and contrasting agents.

In another aspect, the present invention relates to a process of making a polymeric material associated with non-radioactive particles and radioactivating them. The process comprises contacting the polymeric material with a solution of or a colloidal suspension of a non-radioactive particle. In a preferred embodiment, the polymeric material is porous and comprises at least part of the non-radioactive particles within the pores therein. More

preferably, the polymeric material is in microparticle form and is suitable for injection or implantation into a human. In another preferred embodiment, the process comprises a step of heating a metal salt solution containing polymeric material at a temperature and for a time sufficient to associate the metal particles with the polymeric material. In another
5 preferred embodiment, the process further comprises a step of mixing a reducing agent with a metal salt solution or irradiating the mixture with an irradiation source such as ultraviolet light. In a more preferred embodiment of a process of the present invention, the solution is gold chloride (AuCl_3 or HAuCl_4) having a concentration ranging from about 0.01 mg/L to about 5 g/L. Notwithstanding the preceding description, the present invention also
10 embraces a process of making a polymeric material associated with initially radioactive particles, i.e., particles that were radioactive prior to their association with the polymeric material. *See, e.g., Example 13.*

In an embodiment, radioactivating the particles can include subjecting the particles to neutron activation. In an embodiment, a non-radioactive particle can be an inorganic
15 metal compound. In an embodiment, an inorganic metal compound can be a metal oxide.

In yet another aspect, the present invention is directed to a process of making a polymeric material associated with radioactive particles, e.g., of metals or metal compounds, which comprises contacting a polymeric material with a solution of non-radioactive particles, e.g., colloidal metal or colloidal metal compound, and radioactivating
20 the particles. Preferably, the polymeric material is porous and comprises at least part of the colloidal particles within the pores therein. In another preferred embodiment, the polymeric material is in microparticle form having dimensions ranging from about 40 microns to about 2000 microns in diameter. In yet another preferred embodiment, the process comprises packing polymeric material, preferably, in porous microparticle form, in a
25 column and perfusing the column with a solution of, e.g., a colloidal metal or colloidal metal compound. More preferably for this process, the colloidal particles have diameters that are smaller than the sizes of the pores, as measured by the cross section dimension.

The present invention further relates to a process of making a polymeric material associated with radioactive particles, e.g., of metals or metal compounds, by introducing
30 non-radioactive particles into the initial polymerization solution or suspension of polymeric material, and radioactivating the particles. Preferably, the polymeric material is porous and comprises at least part of the particles within the pores therein.

According to this process, the non-radioactive or radioactive particles, e.g., of metals or metal compounds, can be introduced either as colloidal solutions or as salt solutions. The process further enables colloidal particles that are larger than the pores of the polymeric material to be trapped within the pores, resulting in particles that are more tightly attached to the polymers. In a specific embodiment, the initial polymerization solution or suspension for the polymeric material comprises N-(tris(hydroxymethyl)methyl)acrylamide, 2-(N,N-diethylamino)ethylacrylamide, and N,N'-methylenebisacrylamide.

In another aspect, the present invention provides an injectable composition that comprises polymeric microparticles associated with particles, e.g., of metals or metal compounds, and a biocompatible carrier. In a preferred embodiment, the injectable composition comprises microparticles that are porous and having at least part of the particles of metals or metal compounds deposited within the pores therein.

In another preferred embodiment of the injectable composition, the microparticles comprise one or more polymers selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones, styrenics, and polysaccharides. In yet another preferred embodiment, the microparticles comprise an elastomer, a hydrogel, a water swellable polymer, or combinations thereof.

In another preferred embodiment, the injectable composition comprises microparticles that are substantially spheric microspheres suitable for radiation therapy or embolization. More preferably, the microspheres comprise a hydrogel associated with colloidal gold particles and are suitable for injection or implantation into a human. In a most preferred embodiment, the microspheres have diameters ranging from about 10 μm to about 2000 μm , useful for embolization, comprise at least 50% water by weight, and comprise a hydrophilic acrylic copolymer comprising, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge. Further, the microspheres may also comprise one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

In yet another aspect, the present invention provides a method of prophylactic or therapeutic treatment of a mammal, which comprises administering to said mammal polymeric microparticles associated with radioactive particles, e.g., of metals or metal compounds. In a preferred embodiment, the administration is by means of injection through a syringe or a catheter. The method of treatment encompassed by the present invention includes radiation therapy and embolization.

The present invention further provides a kit for performing a prophylactic or therapeutic treatment of a mammal. The kit comprises a sterile container and sterile and biocompatible polymeric microparticles associated with radioactive particles, e.g., of metals or metal compounds. In another embodiment, the present invention provides a kit for performing a prophylactic or therapeutic treatment of a mammal that comprises a needle or a catheter, means for injecting a liquid based composition through said needle or catheter, and sterile and biocompatible polymeric microparticles associated with particles of metals or inorganic metal compounds.

In an embodiment, a method of treating a neoplasm can include administering radioactive metal or metal compound labeled microparticles to a subject, the microparticles embolizing a blood vessel supplying blood to the neoplasm, and the microparticles delivering a dose of radioactivity is delivered to the neoplasm, thereby treating the neoplasm. In an embodiment, the dose of radioactivity can be sufficient to kill the neoplasm. In an embodiment, the dose of radioactivity can be sufficient to prevent recanalization of a blood vessel supplying blood to the neoplasm. In an embodiment, the neoplasm can be liver cancer.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 depicts a micrograph of colloidal gold-associated microspheres before neutron activation.

FIG. 2 depicts a micrograph of colloidal gold-associated microspheres after neutron activation.

DETAILED DESCRIPTION

The present invention provides a unique and valuable system useful for labeling, controlling, and tracking implantable or injectable polymeric materials, especially microparticles, that are used *in vivo*, especially in humans, for prophylactic and/or

therapeutic purposes. The microparticles can include radioactive isotopes for delivering radiation therapy to tissues. Specifically, the invention allows physicians, *e.g.*, surgeons and radiologists, to safely and effectively control and track the labeled materials during and after administration into the body. Therefore, the invention provides polymeric materials, especially microparticles, that are associated with radioactive metal or metal compound particles, especially radioactive colloidal gold particles, which are visible under regular light through naked eye and optionally detectable by radio imaging and/or magnetic resonance imaging instruments.

The invention also provides methods and processes of associating the polymeric materials, especially porous polymeric materials, with radioactive particles. The invention further provides injectable solutions and kits that comprise polymeric microparticles associated with radioactive particles. Moreover, the invention provides methods of prophylactic and/or therapeutic treatment of various conditions in a mammal by administering to the mammal microparticles associated with radioactive particles, *e.g.*, metals or metal compounds.

Small particles are commonly used for the internal delivery of therapeutically useful amounts of radiation to tumors. The internal delivery of radioactivity, compared to external delivery by radioactive sources or beams, allows the use of less penetrating radioactive sources. Useful radioactive isotopes for internal radiotherapy usually emit beta particles or soft x-rays, but other sources, such as alpha- and Auger electron-emitting isotopes, have also been proposed. Administering a weakly penetrating radiation source directly to a tumor allows a very large radiation dose to be delivered to the tumor, while minimizing radiation damage to healthy tissue.

Probably the most commonly used internal radiation delivery vehicles are small metal capsules ("seeds"), which either contain radioactive material or are themselves radioactive. The seeds are implanted directly into the tumor by means of a catheter or syringe. For example, such devices are used in prostate brachytherapy.

Particulate material has long been employed as a delivery vehicle for internal radiotherapy. The intravenous injection of either insoluble radioactive zinc sulfide, or radioactive gold adsorbed onto an inert microparticulate carbon carrier, are described in the earliest reports on localized radiotherapy (Muller and Rossier, *Acta Radiologica*, 1951, vol. 35, p. 449). Since then, a wide variety of radioactive microparticles has been described, including suspensions or colloids of insoluble radioactive inorganic particles, and ceramic

or polymeric microparticles impregnated with radioactive material (Ercan, in *Microspheres, Microcapsules, and Liposomes*, Vol 2 (Citius Books, 1999)).

Microparticles can be administered to a subject for a variety of purposes. One such purpose is to cause embolization of blood vessels. Embolization can block or impede the flow of blood from or to an area of tissue. Neoplasm therapy is a typical application of embolization. Blood vessels supplying blood to the neoplasm, such as a tumor, can be embolized, thus depriving the tumor of its blood supply and thereby promoting tumor death. In particular, liver cancer, such as hepatocellular carcinoma, can be treated and/or palliated using this technique.

Chemotherapy has also found widespread use in therapy of a large variety of neoplasms. The drugs and applications thereof will be well known to one of ordinary skill in the art. Combination of chemotherapy with embolization radiation therapy has been reported to provide palliation of hepatocellular carcinoma (Ramsey DE et al, "Chemoembolization of hepatocellular carcinoma," *J Vasc Interv Radiol* 13(9 Suppl):S211-21, 2002).

Radiation therapy is another technique commonly employed for neoplasm therapy. Radiation is typically delivered to tissue by a variety of methods, such as exposure of a subject to an external source, implantation of radioactive "seeds" into the subject, intravascular administration, and parenteral administration, among others. Radiation has been administered in the form of radioactive metal particles (Muller and Rossier, "A new method for the treatment of cancer of the lungs by means of artificial radioactivity," *Acta Radiologica* 35: 449-468, 1951), and as microspheres of glass, ceramic, albumin, and certain polymers containing radioisotopes ^{99m}Tc , ^{90}Y , ^{188}Re , ^{32}P , and ^{166}Ho (Nijsen J.F.W. et al, "Advances in nuclear oncology: microspheres for internal radionuclide therapy of liver tumours," *Curr Med Chem* 9(1):73-82, 2002).

Some disadvantages of present radioactive microsphere techniques derive from the undesirable properties of the microspheres. For example, glass and ceramic microspheres are dense and thus form poor suspensions that are difficult to make uniform and are difficult to administer by catheter, which is the preferred method. They are heavy and tend to sediment, impeding flow. They can also be difficult to detect after administration because they are not radiopaque or visible on magnetic resonance imaging. Materials such as glass, ceramic, protein, and polymer can be damaged by exposure to radiation. The damage can cause disintegration, etching, crosslinking, aggregation, and other changes

which can destroy the uniformity of the microspheres and their physical or chemical properties. In addition, materials such as glass, ceramic, and many solid polymers cannot be substantially deformed without fracture, making the delivery of large particles through a catheter or needle difficult.

5 In a preferred embodiment, the present invention provides a microsphere including a porous hydrogel associated with radioactive colloidal gold particles. The microsphere can be manufactured, for example, by first making a hydrogel infused with nonradioactive colloidal gold particles (^{197}Au), and then radioactivating the gold (to ^{198}Au , half life about 65 hours) by neutron bombardment. The hydrogel can be infused with other materials, such
10 as a metal or a metal compound. For example, stable ^{165}Ho can be associated with the hydrogel and then radioactivated (to ^{166}Ho , half life about 27 hours).

A variety of neutron sources can be used for radioactivation of stable isotopes by neutron activation, such as reactors, accelerators, and radioisotopic neutron emitters. Systems and methods for neutron activation are described above and in, e.g., U.S. Patents
15 Nos. 6,149,889, incorporated herein by reference, and 6,328,700. Nuclear reactors with their high fluxes of neutrons from uranium fission can offer the highest available sensitivities for most elements. Different types of reactors and different positions within a reactor can vary considerably with regard to their neutron energy distributions and fluxes due to the materials used to moderate (or reduce the energies of) the primary fission
20 neutrons. However, most neutron energy distributions are quite broad and include three principal components (thermal, epithermal, and fast).

The thermal neutron component includes low-energy neutrons (energies below 0.5 eV) in thermal equilibrium with atoms in the reactor's moderator. At room temperature, the energy spectrum of thermal neutrons is best described by a Maxwell-Boltzmann
25 distribution with a mean energy of 0.025 eV and a most probable velocity of 2200 m/s. In most reactor irradiation positions, 90-95% of the neutrons that bombard a sample are thermal neutrons. In general, a one-megawatt reactor has a peak thermal neutron flux of approximately $1\text{E}13$ neutrons per square centimeter per second.

The epithermal neutron component includes neutrons (energies from 0.5 eV to about
30 0.5 MeV) which have been only partially moderated. A cadmium foil 1 mm thick absorbs all thermal neutrons but will allow epithermal and fast neutrons above 0.5 eV in energy to pass through. In a typical unshielded reactor irradiation position, the epithermal neutron

flux represents about 2% the total neutron flux. Both thermal and epithermal neutrons induce (n,gamma) reactions on target nuclei.

The fast neutron component of the neutron spectrum (energies above 0.5 MeV) includes the primary fission neutrons which still have much of their original energy following fission. Fast neutrons contribute very little to the (n,gamma) reaction, but instead induce nuclear reactions where the ejection of one or more nuclear particles - (n,p), (n,n'), and (n,2n) - are prevalent. In a typical reactor irradiation position, about 5% of the total flux consists of fast neutrons.

Alternatively, previously neutron-activated gold can be associated with a hydrogel. These embodiments, along with others, overcome a number of disadvantages of foregoing systems. For example, stable gold atoms (^{197}Au) have a high cross-section for neutron capture and efficiently transmutes to ^{198}Au . The efficient radioactivation requires comparatively brief exposure to the neutron source, thereby reducing damage to the hydrogel. The hydrogel, being mostly water, has a density approaching that of water, so a hydrogel microsphere can form a uniform suspension that is readily administered by catheter. The radioactive gold, in addition to emitting beta particles for therapeutic application, also emits gamma rays, which can be readily detected, such as by a gamma camera, thereby facilitating visualization after administration. The amount of radiation being delivered to a target, such as a tumor, as compared to non-target tissue, can also thereby be measured, which is important for ensuring that sufficient radiation reaches the target without unduly exposing non-target tissue.

The amount of radiation delivered to a target by radioisotope-labeled microspheres can be controlled in a variety of ways, as by varying the amount of radioisotope associated with the spheres; the extent of radioactivation of the element; the quantity of microspheres administered; and the size of microspheres administered.

Radiation can be delivered in amounts sufficient to cause death of the target tissue. Radiation can also be delivered in amounts sufficient merely to prevent recanalization of target tissue. To prevent recanalization, sufficient radiation is delivered to provoke a scarring response in, for example, surrounding capillaries, without so damaging the tissue to kill it. The scarring response helps inhibit the formation of new blood vessels, thereby enfeebling neovascularization stimuli produced by a tumor.

As used in the present invention, the term "implant" means a substance that is placed or embedded at least in part within the tissue of a mammal. An "implantable"

substance is capable of being placed or embedded within the tissue through whatever means. For example, within the meaning of the present invention, a piece of traditional prosthetic device is an implant. So are substances, such as microparticles, that are placed within the dermal tissue of a mammal.

5 As used in the present invention, the term “embolization” means the occlusion or blockage of a blood vessel. The occlusion or blockage may occur either due to blood clots or emboli as a result of a physiological condition or due to an artificial act of embolic materials. In this regard, according to the present invention, an embolus is different from an implant.

10 As used in the present invention, the term “hydrogel” refers to a polymeric composition, comprising at least 50% water by weight, and can comprise a wide variety of polymeric compositions and pore structures.

 As used in the present invention, the term “injectable” means capable of being administered, delivered or carried into the body via a needle, a catheter, or other similar
15 ways.

 As used in the present invention, “microparticles” means polymer or combinations of polymers made into bodies of various sizes. The microparticles can be in any shape, although they are often in substantially spherical shape, in which case the microparticles are referred to as “microspheres” or “microbeads.”

20 “Substantially spherical,” as used in the present invention generally means a shape that is close to a perfect sphere, which is defined as a volume that presents the lowest external surface area. Specifically, “substantially spherical” in the present invention means, when viewing any cross-section of the particle, the difference between the average major diameter and the average minor diameter is less than 20%, preferably less than 10%. The
25 microspheres of the present invention may comprise, in addition to the particles, other materials as described and defined herein.

 As used in the present invention, “associated with” means the condition in which two or more substances having any type of physical contact. For example, when a polymeric material is “associated with” metal or metal compound particles, the metal
30 particles may be deposited on the surface of the polymeric material, within the material, or, if the material is porous, within the pores of the material, through any type of physical or chemical interactions such as through covalent bond, ionic bond, or van der Waal’s bond, or through impregnating, intercalating, or absorbing. According to the present invention,

when a polymeric material is associated with metal or metal compound particles, it is “labeled” with the metal or metal compound particles.

Polymeric Materials Comprising Particles of a Metal or Metal Compound

5 In one aspect, the present invention is directed to a polymeric material that comprises colloidal metal particles. The polymeric material of the present invention includes synthetic and natural polymers. Preferably, the polymeric material is porous synthetic polymeric material and comprises at least part of the colloidal metal particles within the pores therein. In a preferred embodiment of the present invention, the material
10 comprises one or more polymers selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones, styrenics, and polysaccharides. In another preferred embodiment, the polymeric material of the present invention is or is made to be an elastomer, a hydrogel, a water swellable polymer, or combinations thereof.

15 According to the present invention, the metal containing polymeric materials may be used in any medical applications, but they are especially suitable as implantable and/or injectable devices. In a more preferred embodiment of the present invention, the colloidal metal labeled polymeric material is in microparticle form and useful as emboli for prophylactic or therapeutic embolizations. Therefore, the polymeric materials of the
20 present invention are particularly suitable in injectable implantations or embolizations as small particles, such as microparticles, microbeads or microspheres. These microparticles are usually difficult to detect after injection into the body. In a preferred embodiment of the present invention, the microparticles are rendered detectable by radiological means, for example, by gamma camera.

25 Many types of polymeric microparticles or microspheres, either for tissue bulking, dermal augmentation, radiation therapy, or embolization purposes, are suitable for the present invention. For example, the microparticles disclosed in U.S. Patent Nos. 4,657,553; 4,999,188; 5,007,940; 5,092,883; 5,344,452; 5,571,182; 5,635,215; 5,648,100; 5,785,997; 5,798,096; 5,995,108; 6,328,700; 6,335,028; and 6,436,424 are encompassed by the present
30 invention as polymeric materials that can be associated with colloidal metal particles according to the present invention. The above U.S. patents are herein specifically incorporated by reference. Also incorporated by reference are United States Patent Application Serial Nos. 09/419,114; 09/528,989; and 09/528,991; PCT applications

PCT/IB01/01266; PCT/US01/09618; PCT/US01/08258; PCT/US01/09619; and Japanese laid open patent application 6-56676.

In a preferred embodiment of the present invention, the polymeric material comprises an acrylic polymer. Because of its wide applications in medical implantable devices, polymethacrylates such as poly(methyl methacrylate) and poly (2-hydroxyethyl methacrylate) are especially suitable for the present invention.

In another preferred embodiment of the present invention, the porous polymeric materials comprise microbeads or microparticles based on a biocompatible, hydrophilic, substantially spherical, and non-toxic polymers. The microspheres are injectable and/or implantable and not capable of being digested or eliminated through the mammal's immune or lymphatic system. More preferably, the hydrophilic copolymers usable for this application are those of the acrylic family such as polyacrylamides and their derivatives, polyacrylates and their derivatives as well as polyallyl and polyvinyl compounds. All of these polymers are preferably crosslinked so as to be stable and non-resorbable.

In a particularly preferred embodiment of the present invention, the microparticle comprises a polymeric material that comprises a hydrophilic acrylic copolymer, which contains, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50% by weight of one or more monomers having a cationic charge. More preferably, the neutral hydrophilic acrylic monomer is selected from the group consisting of acrylamides, methacrylamides and hydroxymethylmethacrylate; the difunctional monomer is selected from the group consisting of N,N'-methylene-bis-acrylamide, N',N'-diallyltartradiamide, and glyoxal-bis-acrylamide; and the monomer having a cationic charge is a monomer that has a tertiary and/or quaternary amine function.

In addition, the microparticle may optionally comprise one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

In another particularly preferred embodiment of the present invention, the polymeric material comprises poly (vinyl alcohol). Polyvinylalcohol particles are the most common material used to date in a variety of embolization applications. WO 00/23054, the content of which is incorporated by reference, discloses substantially spherical shaped microspheres

comprising cross-linked PVA. The microspheres described therein has certain advantages in embolization. For example, due to their spherical shape or substantially spherical shape, microspheres can properly and completely occlude artery lumen because they can establish complete contact with all the surface of the artery which is cylindrical. In addition, the
5 microspheres can be easily calibrated, and samples or suspensions containing these microspheres will not block or clog catheters because they always have the same dimension regardless of their space orientation in the catheter. The invention described herein encompasses PVA microspheres useful for radiation therapy and/or embolization. The PVA microspheres preferably comprise crosslinked polyvinylalcohol.

10 Preferred diameters for the microspheres depend on the type of embolization to be performed and can be readily determined by a skilled artisan. In a preferred embodiment, the present invention encompasses microspheres, which comprise in crosslinked and hydrogel form, from about 0.5% to about 20% cross-linked poly(vinyl alcohol) by weight. In other embodiments, the crosslinked polyvinylalcohol microspheres may further comprise
15 one or more of a cell adhesion promoter or a marking agent other than the colloidal metal.

The polymeric material of the present invention, when in microparticle form, preferably have dimensions ranging from about 1 μm to about 2000 μm . Preferably, the microparticles are substantially spherical microspheres with diameters ranging from about 10 μm to about 2000 μm , more preferably, from about 40 μm to about 1200 μm .

20 According to a preferred embodiment of the present invention, the polymeric material contains or is made to contain pores. Preferably, the material comprises pores both on the surface and within. The pores contained within the polymeric material of the present invention have sizes, measured in cross-section diameters, ranging from about 1 nm to about 10 μm and, preferably, from about 1 nm to about 1000 nm. The lengths of the pores
25 vary depending on the dimensions of the material. The pores facilitate the impregnation of and actually contain the colloidal metal particles, which are preferably trapped within the pores.

The porous polymeric material of the present invention preferably contains within the pores radioactive or non-radioactive particles that have dimensions ranging from about
30 1 nm to about 1000 nm, more preferably, from about 1 nm to about 500 nm. The present invention contemplates preferably particles of phosphorus, yttrium, iodine, gold, rhenium, or holmium. However, other particles can be included, as described above. The particle can be, e.g., gold, rhenium, holmium, silver, platinum, copper, titanium and chromium, and

their inorganic compounds. The impregnation of the particles of metals or inorganic metal compounds within the polymers are the results of either direct deposition of the particles on the porous polymeric material or a reduction or oxidation process from a metal salt solution.

5 In a particularly preferred embodiment, the present invention provides a substantially spherical microparticle, or a microsphere, which comprises a hydrogel associated with colloidal gold particles, wherein the microsphere is suitable for injection or implantation into a human. In a more preferred embodiment, the present invention provides a microsphere having a diameter ranging between about 10 μm and about 2000 μm , useful
10 for embolization, which comprises a hydrophilic acrylic copolymer associated with colloidal gold particles, wherein the hydrophilic acrylic copolymer comprises, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge.

15 The microsphere of the present invention may also comprise one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents. Further, the microsphere may optionally comprise a marking agent selected from the group consisting of dyes, imaging
20 agents, and contrasting agents.

Processes Of Associating Polymeric Materials With Colloidal Metal Particles

Another aspect of the present invention relates to processes of associating metal or metal compound particles with the polymeric material. According to the present invention,
25 the association process can be accomplished in at least three ways. First, the particles can be associated with the polymeric materials in a chemical reaction with a salt solution. Second, the particles can be deposited on and/or within the polymeric material through direct contact between the material and a colloidal solution of the particles. Third, the metal containing polymeric material can be produced by introducing a metal salt or
30 colloidal solution into the initial polymerization solution or suspension of the polymeric material. In all three methods, the metal or metal compound particles may be radioactive before its incorporation into the polymeric material. On the other hand, the metal or metal compound particles may be non-radioactive before its incorporation into the polymeric

material. Also, in all three methods, the colloidal metal particles are preferably permanently associated on the polymeric materials, enable better detection and control of such materials in implantation applications. The various polymeric materials mentioned above are suitable for the association processes of the present invention.

5 According to the present invention, colloidal metal particles can be associated with a polymeric material by contacting the polymeric material with a metal salt solution for a time and at a temperature sufficient to reduce the metal salt into metal particles that are deposited on or within the polymeric material. In a preferred embodiment of the present invention, the polymeric material is porous and that the process enables the porous
10 materials to comprise at least part of the colloidal metal particles within the pores of the material. In such cases, the sizes of the metal particles may either be larger or smaller than the sizes of the pores of the material, as measured by the cross-sections of the pores.

The associating process, according to the present invention, can be accelerated by heating the metal salt solution. The process can be further accelerated by the addition of a
15 reducing agent. Any agent that is known to have the ability to reduce a metal salt into metal particles can be used for this purpose. Preferred reducing agents include sodium citrate, ascorbic acid, phosphorous derivatives, tannic acid, citric acid, and combinations thereof. Another way of accelerating the reduction process is irradiation of the mixture of the polymeric material and the metal salt solution. Preferred sources of irradiation include
20 ultraviolet light such as that from a mercury lamp. After the impregnation/deposition processes, the polymeric material is preferably washed and/or filtered with water or saline to remove any non-deposited materials.

In a preferred process embodiment, the metal salt solution is gold chloride (such as HAuCl_4 or AuCl_3) having a concentration ranging from about 0.01 mg/L to about 5 g/L.
25 More preferably, the process comprises heating the gold chloride solution containing the polymeric material. Further, the addition of a reducing agent could accelerate the impregnation process, so could irradiation from source such as ultraviolet light, as discussed above.

The present invention also provides a process of associating colloidal particles of
30 metals or metal compounds with a polymeric material by contacting the polymeric material with a colloidal solution of metal or metal compound particles. In a preferred embodiment of the present invention, the polymeric material is porous and that the process enables the porous materials to comprise at least part of the colloidal particles within the pores of the

material. In such a process, the sizes of the colloidal metal particles are preferably smaller than the sizes of the pores, as measured by the dimension of the cross sections of the pores.

In another preferred embodiment, the polymeric material is in microparticle form and has dimensions ranging from about 1 μm to about 2000 μm . A more preferred process for this direct deposition of colloidal particles comprises packing the polymeric material, such as microparticles, in a column and perfusing the column with the colloidal metal solution. This process can be preferably followed by rinsing the column with water or saline. When colloidal particles are used for porous materials, the particles are preferably of sizes smaller than the pores of the polymeric material. They also should be preferably suspended with a surfactant to maintain in a dissociated form.

According to the present invention, another process of associating colloidal particles of metal or metal compound with the polymeric material comprises adding the colloidal particles or a metal salt solution into the initial polymerization solution or suspension for the polymeric material. In a preferred embodiment of the present invention, the resultant polymeric material is porous and that the process enables the porous materials to comprise at least part of the colloidal metal particles within the pores of the material.

In such a polymerization/association process, there is preferably no change in the polymerization process for the polymeric material itself. Therefore, any polymerization process that produces a polymeric material can be incorporated into the process of the present invention by adding a solution of metal salt or colloidal metal into the initial polymerization solution or suspension. For example, polymerization processes disclosed in references incorporated herein are encompassed by the present invention. In particular, polymerization processes disclosed in U.S. Patent No. 5,635,215 for producing acrylic microspheres, and in WO 00/23054 for producing PVA microspheres can be incorporated into the process of the present invention to produce hydrophilic acrylic microspheres or PVA microspheres containing colloidal particles. When the initial polymerization solution or suspension is transformed into a acrylic or PVA microsphere, preferably in hydrogel form, the colloidal particles are trapped within the polymer network and cannot be released any longer. In this case they are located inside the polymer pores. In case of a porous polymeric material, the resulting metal containing material from this process may contain colloidal metal particles that are larger in size than the sizes of the pores, as measured by the dimensions of the cross sections of the pores.

Injectable Compositions, Kits, and Methods of Use

The present invention further encompasses injectable compositions, kits, and methods of use in connection with the colloidal metal containing polymeric materials disclosed above.

5 In one embodiment, there is provided an injectable composition that comprises polymeric microparticles associated with colloidal metal particles and a biocompatible carrier. The various embodiments of the colloidal metal containing microparticles disclosed herein are suitable for the injectable compositions. In addition, the microparticles and biocompatible carriers disclosed in the various U.S. patents, U.S. and PCT patent
10 applications incorporated by references herein are also suitable for the injectable compositions of the present invention.

In another embodiment, the present invention provides a method of prophylactic or therapeutic treatment of a mammal, preferably a human, which comprises administering to the mammal polymeric microparticles associated with radioactive particles. Due to the
15 unique characters of the microparticles of the present invention, the administration is capable of being well controlled and/or manipulated both before and after the process, as the microparticles are readily visible under regular light before the administration and optionally using radio-imaging and/or magnetic resonance techniques after the administration.

20 Suitable treatment encompassed by the present invention includes radiotherapy, dermal augmentation, tissue bulking, embolization, drug delivery, and treatment of gastroesophageal reflux disease, urinary incontinence, and vesicoureteral reflux disease. The administration according to the method of treatment of the present invention is preferably carried out by means of injection through a syringe or a catheter. In this regard,
25 the methods of treatment disclosed in the U.S. patents, U.S. and PCT patent applications incorporated by reference herein are also encompassed by the present invention's methods. Finally, the present invention provides a kit for performing a prophylactic or therapeutic treatment of a mammal. The kit preferably comprises a sterile container and sterile and biocompatible polymeric microparticles associated with colloidal metal particles. In
30 another preferred embodiment, the kit of the present invention for performing a prophylactic or therapeutic treatment of a mammal comprises a needle or a catheter; means for injecting a liquid based composition through said needle or catheter; and sterile and biocompatible polymeric microparticles associated with colloidal metal particles. In this

regard, the various embodiments of the microparticles disclosed herein and the various embodiments disclosed in the U.S. patents, U.S. and PCT patent applications incorporated by reference herein are also encompassed by the present invention's kit.

The present invention is further described by reference to the following examples that detail the preparation of colloidal metal labeled microparticles. In addition, the examples disclosed in the U.S. patents and U.S. and PCT patent applications incorporated by reference herein are also illustrative of the present invention. The examples should in no way be construed to limit the scope of the present invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the purpose and scope of this invention.

EXAMPLES

Example 1 Gold staining of embolic spherical material constituted of a synthetic polymer containing crosslinked collagen (e.g., Embosphere® microspheres)

Solutions of HAuCl_4 (0.1 to 5.0 g/L) (Solution I) and of sodium citrate as reducing agent (1 % by weight) (Solution II) were prepared. A suspension of Embosphere® microspheres (10 mL) and Solution I (20 mL of the desired concentration) were heated to boiling and then 2 mL of Solution II was added. After 10 minutes the solution and Embosphere® suspension turned to red, indicating the formation of gold colloidal particles within the solid material network. The beads were then filtered and washed several times with water and saline. Similar results were obtained when using other reducing agents, instead of sodium citrate, such as ascorbic acid, phosphorous derivatives or sodium citrate and tannic acid.

Example 2 Gold staining of PVA particles (spherical or irregular) as embolic material

Solutions of 3 g/L of HAuCl_4 (Solution I) and of 1% ascorbic acid as reducing agent (Solution II) were prepared. 10 mL of a suspension of PVA solid particles was mixed with 20 mL of solution and heated to boiling. To the boiling suspension, 2 mL of Solution II was added. After 10 minutes, the suspension of embolic material turned to red, indicating the formation of gold colloidal particles within the solid material network. The beads were then filtered and washed extensively with water and saline. Similar results were obtained using other reducing agents, instead of ascorbic acid, such as citric acid, tannic acid, and phosphorous derivatives.

Example 3 Embolic solid material staining without reducing agents

The same procedure was used as described in Example 1, but without a reducing agent. The suspension of Embosphere® microspheres or PVA particles with Solution I were heated to boiling for an extensive period of time (15 minutes or more). The beads and the solution appeared red-brown, which confirmed the formation of gold particles within the solid material network. The beads were then treated with the same manner as described in Examples 1 and 2. The reduction of gold could also be accomplished by irradiation of the samples with a mercury lamp for about 48 hours at 25°C.

Example 4 Staining procedure concomitant to bead preparation by acrylic polymerization

In a beaker containing 100 mL of HAuCl_4 solution at a concentration of 3 g/Liter, 29 g of sodium chloride and 13.5 g of sodium acetate were dissolved. 200 mL of glycerol was added and then the pH was adjusted between 5.9 and 6.1. Then 45 g of N-tris-hydroxy-methyl-methylacrylamide, 17.5 g of diethylaninoethylacrylamide and 5 g of N,N-methylene-bis-acrylamide were added. Once the solution was at 60°C, 60 mL of a water solution containing 10 g of gelatin was added. The total volume of the mixture was adjusted to 500 mL by addition of hot water. To this solution 10 mL of a 700 mg ammonium persulfate solution and 2 mL of N,N,N',N'-tetramethylenediamine were added. The resulting mixture was rapidly stirred to mix all ingredients together and poured into double volume of stirred paraffin oil at 58°C. After a few minutes, the polymerization reaction of acrylic monomers was manifested by an increase of temperature. To the emulsion 400 mL of sodium citrate solution (1% by weight) was then added and the suspension heated to 70-80°C. Resulting red beads were recovered by decanting, washed carefully, sieved and sterilized in an autoclave in a physiological saline medium.

Example 5 Staining procedure concomitant to bead preparation by crosslinking

To an aqueous solution of PVA (50 g in 300 mL), glutaraldehyde (10 ml of 25% aqueous solution) and HAuCl_4 solution (100 mL of 3 g/L) were added under stirring at 55°C. This solution was then dispersed in a medium consisting of 1000 mL of paraffin oil and 1 mL of Arlacel®. Thionyl chloride (10 mL) was then introduced to the emulsion and kept at 25°C under stirring (180 rpm) for five hours. To the suspension, 400 mL of a

solution of sodium citrate (1% by weight) was then added and the mixture heated at 70-90 C for one hour. Resulted crosslinked PVA microsphere were recovered by decanting. They were washed, sieved and sterilized in an autoclave in a saline medium.

5 **Example 6 Staining of beaded embolic agent with colloidal platinum**

 A solutions of H_2PtCl_6 at a concentration of 5.3 g/L was prepared in water under stirring (Solution I). A second solution of saturated hydrazine sulfate in water was also prepared (Solution II). To a suspension of 10 mL of embolic beads (e.g., Embosphere® beads) 20 mL of Solution I was added under stirring. The resulting suspension was then
10 heated to boiling temperature and then added with 5 mL of Solution II. After 10 minutes agitation, the embolic materials turned to gray, indicating the formation of colloidal platinum nanoparticles. The beads were then filtered and washed several times with water and physiological saline.

15 **Example 7 Staining of a commercially available embolic material**

 The same procedure was used as described in Example 1, but Ivalon® was used instead of Embosphere® microspheres. The suspension of Ivalon® irregular particles with Solution 1 (HAuCl_4 , 3 g/L) was heated to boiling temperature and then 2 mL of Solution II (1% sodium citrate in water) was added. After 10 minutes of agitation, the suspension
20 turned to red-brown, indicating the formation of gold colloidal particles in the Ivalon® particles. The particles were then filtered and washed several times with water and saline. Similar results were obtained when using other reducing agents instead of sodium citrate such as ascorbic acid, phosphorous derivatives or sodium citrate/tannic acid. The reduction to colloidal gold could also be made by irradiation of the suspension with a mercury lamp
25 for about 48 hours at 25°C.

Example 8 Staining of embolic biodegradable embolic particles

 This process applies to embolic microparticles (irregular arid spherical) composed of polysaccharide and/or proteins (e.g., albumin). The same procedure was used as
30 described in Example 1, but biodegradable solid embolic material is used instead of Embosphere® microspheres. 10 mL particles were put in suspension with 20 mL of an aqueous Solution I of HAuCl_6 at 3 g/L. The mixture was then heated to boiling temperature

and then 2 mL of 1% sodium citrate solution in water was added. After 10 minutes agitation, the suspension turned to red-brown indicating the formation of gold colloidal particles inside the embolic material. The particles were then filtered and washed several times with water and saline. Similar results were obtained using other reducing agents, instead of sodium citrate, such as ascorbic acid, phosphorous derivatives or sodium citrate/tannic acid. The reduction to colloidal gold could also be made by irradiation of the suspension with a mercury lamp for up to about 48 hours at 25°C.

Example 9 Staining of solid embolic material with gold colloidal particles

This method of staining applies to embolic material that has porous structure with pores larger than 10 nm in diameter. Embolic material in aqueous suspension was packed in a glass column. Through the column a colloidal solution of gold was perfused. Colloidal particles that had a size smaller than the pores of the solid embolic material were trapped within the embolic pore network. The excess of gold colloidal particles or colloidal particles that were larger than the pores of the solid embolic were washed out the column by means of a physiological buffer. After the treatment the embolic material showed a red like color, indicating the presence of colloidal gold entrapped within the pore network.

Example 10 Injectable compositions containing gold labeled Embosphere® microspheres

Gold labeled Embosphere® microspheres, as described in Examples 1, 3 and 4 are washed with normal saline and then sterilized by autoclave. The resultant microspheres are mixed with non-pyrogenic, sterile, physiological saline in ratios ranging from about 0.05 mL microspheres/mL saline to about 0.5 mL microspheres/mL saline.

Example 11 Kits containing gold labeled Embosphere® microspheres

A total amount of 8 mL of sterile injectable composition as described in Example 10 is transferred, under sterile condition, into a glass vial of 10 mL in capacity and having a stopper sealed by an aluminum cap equipped with a colored tag.

Example 12 Neutron activation of gold labeled Embosphere® microspheres

A sample of commercial EmboGold® microspheres, 40 – 120 µm in diameter, was washed 7 times with sterile water. A sample of the microspheres before neutron activation

was viewed under a microscope (see FIG. 1). Two aliquots, each containing approximately 100 μL of microspheres, were removed and subjected to neutron bombardment for 2 hours in a SLOWPOKE nuclear reactor facility. The neutron flux in the reactor was 5.5×10^{11} neutrons $\text{cm}^{-2} \text{s}^{-1}$. The analysis of the irradiated samples indicated that about 14 μCi of ^{198}Au was produced in each sample, corresponding the presence of about 75 μg of ^{197}Au before the neutron activation experiment. The measured gold content is consistent with an estimate of the expected amount. A sample of the microspheres after neutron activation was viewed under a microscope (see FIG. 2). Comparison of the visual appearance of the microspheres before and after neutron activation suggested that the beads remained intact.

Example 13 Incorporation of radioactive gold into Embospheres

A suspension of commercially available Embospheres in 1.5 M aqueous sodium chloride, -- 1 mL of microspheres in a total volume of about 5 mL -- is treated with up to 50 curies of Au-198, as its trichloride or trinitrate salt. The mixture is heated to 90 $^{\circ}\text{C}$ for 30-90 minutes, and then allowed to cool. The microspheres are washed with 1.5 M aqueous sodium chloride. The microspheres retain greater than 50% of the radioactivity.

Equivalents

The embodiments of the present invention described above are intended to be merely exemplary and those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. All such equivalents are considered to be within the scope of the present invention and are covered by the following claims.

Incorporation by Reference

The contents of all references described herein are hereby incorporated by reference. Other embodiments are within the following claims.

What is claimed is:

1. A microsphere comprising a polymeric hydrogel associated with radioactive particles.

2. The microsphere of claim 1, wherein the polymeric hydrogel comprises one or more polymers selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones, styrenics, and polysaccharides.

3. The microsphere of claim 2, wherein the hydrogel is porous.

4. The microsphere of claim 3, wherein the microsphere comprises at least part of the particles within the pores therein.

5. The microsphere of claim 4, wherein the hydrogel is suitable for implantation into a human.

6. The microsphere of claim 1, wherein the particles comprise an element selected from the group consisting of gold, antimony, lanthanum, samarium, europium, terbium, holmium, ytterbium, lutetium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, scandium, platinum, palladium, copper, titanium, and chromium.

7. The microsphere of claim 1, wherein the particles comprise a beta-emitting element.

8. The microsphere of claim 1, wherein the particles comprise an element selected from the group consisting of phosphorus, yttrium and iodine.

9. The microsphere of claim 7, where the beta-emitting element is a gamma-emitting element.

10. The microsphere of claim 7, wherein the element has a half life in the range of about 20 hours to about 30 days.

11. The microsphere of claim 7, wherein the element has a half life in the range of about 20 hours to about 120 hours.

5 12. A microsphere comprising a polymeric hydrogel associated with particles, the particles comprising a metal or metal compound, wherein the microparticle is suitable for injection or implantation into a human.

10 13. The microsphere of claim 12, wherein the polymeric hydrogel comprises one or more polymers selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones, styrenics, and polysaccharides.

14. The microsphere of claim 13, wherein the hydrogel is porous.

15 15. The microsphere of claim 14, wherein the microsphere comprises at least part of the particles within the pores therein.

16. The microsphere of claim 14, wherein the polymeric material comprises pores both on the surface and within.

20

17. The microparticle of claim 16, wherein the pores have sizes ranging from about 1 nm to about 10 μm .

25 18. The microsphere of claim 12, wherein the polymeric hydrogel comprises a hydrophilic acrylic copolymer.

19. The microsphere of claim 18, wherein the hydrophilic acrylic copolymer comprises, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge.

30

20. The microsphere of claim 19, wherein the neutral hydrophilic acrylic monomer is selected from the group consisting of acrylamides, methacrylamides and hydroxymethylmethacrylate.

5 21. The microsphere of claim 14, wherein the difunctional monomer is selected from the group consisting of N,N'-methylene-bis-acrylamide, N',N'-diallyltartrdiamide, and glyoxal-bis-acrylamide.

10 22. The microsphere of claim 14, wherein the monomer having a cationic charge is a monomer having a tertiary and/or quaternary amine function.

15 23. The microsphere of claim 14, wherein the microsphere further comprises one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

20 24. The microsphere of claim 18, wherein the microsphere further comprises a marking agent selected from the group consisting of dyes, imaging agents, and contrasting agents.

25 25. The microsphere of claim 12, wherein the polymeric hydrogel comprises a polymethacrylate.

25 26. The microsphere of claim 20, wherein the polymeric hydrogel comprises poly(methyl methacrylate) or poly (2-hydroxyethyl methacrylate).

27. The microsphere of claim 12, wherein the polymeric hydrogel comprises cross-linked poly (vinyl alcohol).

30 28. The microsphere of claim 12, wherein the microsphere has dimensions ranging from about 1 μm to about 2000 μm .

29. The microsphere of claim 12, wherein the microsphere is a substantially spherical microsphere have a diameter ranging from about 10 μm to about 2000 μm .

30. The microsphere of claim 12, wherein the microsphere is suitable for
5 therapeutic vascular embolization.

31. The microsphere of claim 12, wherein the microsphere is suitable for in vivo radiation therapy.

10 32. The microsphere of claim 12, wherein the particles comprise an element selected from the group consisting of gold, antimony, lanthanum, samarium, europium, terbium, holmium, ytterbium, lutetium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, scandium, platinum, palladium, copper, titanium, and chromium.

15 33. The microsphere of claim 32, wherein the element is gold.

34. The microsphere of claim 12, wherein the element is phosphorus, yttrium or iodine.

20 35. The microsphere of claim 32, wherein the particles have dimensions ranging from about 1 nm to about 1000 nm.

36. The microsphere of claim 32, wherein the particles have dimensions ranging from about 1 nm to about 500 nm.

25 37. The microsphere of claim 12, wherein the particles comprise a colloidal metal.

38. A microsphere, comprising a hydrogel associated with radioactive colloidal gold particles, wherein the microsphere is suitable for injection or implantation into a
30 human.

39. A microsphere, having a diameter ranging between about 10 μm and about 2000 μm , and comprising a hydrophilic acrylic copolymer associated with radioactive colloidal gold particles, wherein the hydrophilic acrylic copolymer comprises, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge.

40. The microsphere of claim 39, wherein the microsphere further comprises one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

41. The microsphere of claim 39, wherein the microsphere further comprises a marking agent selected from the group consisting of dyes, imaging agents, and contrasting agents.

42. An injectable composition suitable for administration to a human comprising polymeric microparticles associated with radioactive particles and a biocompatible carrier.

43. The injectable composition of claim 42, wherein the microparticles comprise one or more polymers selected from the group consisting of acrylics, vinyls, acetals, allyls, cellulose, polyamides, polycarbonate, polyesters, polyimide, polyolefins, polyurethanes, silicones, styrenics, and polysaccharides.

44. The injectable composition of claim 43, wherein the microparticles are substantially spherical microspheres suitable for one or more of embolization and radiation therapy.

45. The injectable composition of claim 42, wherein the radioactive particles comprise colloidal gold.

46. The injectable composition of claim 42, wherein the microparticles have diameters ranging between about 10 μm and about 2000 μm , and comprise a hydrophilic acrylic copolymer comprising, in copolymerized form, about 25 to about 98%, by weight, of a neutral hydrophilic acrylic monomer, about 2 to about 50%, by weight, of a difunctional monomer, and about 0 to about 50%, by weight, of one or more monomers having a cationic charge.

47. The injectable composition of claim 42, wherein the microparticles further comprise one or more cell adhesion promoters selected from the group consisting of collagen, gelatin, glucosaminoglycans, fibronectin, lectins, polycations, natural biological cell adhesion agents or synthetic biological cell adhesion agents.

48. A method of treatment of a human, comprising administering to said human polymeric microparticles associated with radioactive particles.

49. The method of claim 48, wherein administering comprises injecting the polymeric microparticles in the human through a syringe or a catheter.

50. The method of claim 48, wherein the treatment comprises one or more of embolization, radiation therapy, drug delivery, and treatment of gastroesophageal reflux disease, urinary incontinence, and vesicoureteral reflux disease.

51. The method of claim 48, wherein the radioactive particles comprise colloidal gold.

52. A kit for performing a combined embolization and radiation treatment of a human, comprising:

a sterile container; and
sterile and biocompatible polymeric microparticles associated with radioactive particles.

53. The kit of claim 52, wherein the radioactive particles comprise colloidal gold.

54. A kit for performing a combined embolization and radiation treatment of a human, comprising:

a needle or a catheter;

means for injecting a liquid based composition through said needle or

5 catheter; and

sterile and biocompatible polymeric microparticles associated with radioactive particles.

55. The kit of claim 54, wherein the radioactive particles comprise colloidal gold.

56. A process of making a polymeric material associated with radioactive particles, comprising:

contacting the polymeric material with a metal salt solution or a metal compound salt solution at a temperature and for a time sufficient to reduce and deposit

15 particles on the polymeric material; and

radioactivating the particles.

57. The process of claim 56, wherein the polymeric material is porous and at least a portion of the particles are deposited within the pores therein.

58. The process of claim 56, further comprising a step of heating the salt solution to a temperature and for a time sufficient to reduce and deposit metal particles on the polymeric material.

59. The process of claim 56, further comprising a step of adding a reducing agent to the salt solution.

60. The process of claim 56, wherein the salt solution comprising gold chloride and having a concentration ranging from about 0.1 g/L to about 5 g/L.

61. The process of claim 56, wherein the polymeric material is in microparticle form and suitable for injection or implantation into a human.

62. The process of claim 56, further comprising washing the polymeric material associated with metal particles prior to radioactivating.

63. The process of claim 49, wherein the metal or metal compound is selected from the group consisting of gold, antimony, lanthanum, samarium, europium, terbium, holmium, ytterbium, lutetium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, scandium, platinum, palladium, copper, titanium, chromium, and a metal oxide.

64. The process of claim 56, wherein the metal or metal compound is selected from the group consisting of phosphorus, yttrium and iodine.

65. The process of claim 56, wherein radioactivating comprises neutron activating.

66. A process of making a polymeric material associated with radioactive colloidal metal particles, comprising:

contacting the polymeric material with a colloidal metal solution; and
radioactivating the metal particles.

67. The process of claim 66, wherein the polymeric material is in microparticle form and suitable for injection or implantation into a human.

68. The process of claim 67, further comprising:
packing the microparticles in a column; and
perfusing the column with the colloidal metal solution.

69. The process of claim 67, wherein the microparticle is porous and at least part of the colloidal metal particles are deposited with the pores therein.

70. A process of making a polymeric material associated with radioactive colloidal metal particles, comprising:

mixing the colloidal metal particles with the initial polymerization solution or suspension for the polymeric material; and
radioactivating the metal particles.

71. The process of claim 70, wherein the polymeric material is porous and at least part of the colloidal metal particles are deposited with the pores therein.

5 72. The process of claim 70, wherein the polymeric material is in microparticle form and suitable for injection or implantation into a human.

73. The process of claim 70, wherein the initial polymerization solution or suspension for the polymeric material comprises N-tris-hydroxy-methyl-methylacrylamide,
10 diethylaninoethylacrylamide, and N,N-methylene-bis-acrylamide.

74. A process of making a polymeric material associated with radioactive colloidal metal particles, comprising:

 mixing a metal salt solution with the initial polymerization solution or
15 suspension for the polymeric material, and
 radioactivating the metal particles.

75. A method of treating a neoplasm, comprising:
 administering radioactive microspheres to a subject, the microspheres each
20 comprising a porous polymeric hydrogel and radioactive particles deposited in the pores,
 the microspheres embolizing a blood vessel supplying blood to the neoplasm, and the
 microspheres delivering a dose of radioactivity to the neoplasm, thereby treating the
 neoplasm.

25 76. The method of claim 75, wherein the dose of radioactivity is sufficient to kill the neoplasm.

77. The method of claim 75, wherein the dose of radioactivity is sufficient to prevent recanalization of a blood vessel supplying blood to the neoplasm.

30 78. The method of claim 75, wherein the neoplasm is liver cancer.

79. The method of claim 75, wherein the radioactive particles comprise a radioactive metal or metal compound.

5 80. The method of claim 79, wherein the metal or metal compound is selected from the group consisting of gold, antimony, lanthanum, samarium, europium, terbium, holmium, ytterbium, lutetium, hafnium, tantalum, tungsten, rhenium, osmium, iridium, scandium, platinum, palladium, copper, titanium, chromium, and a metal oxide.

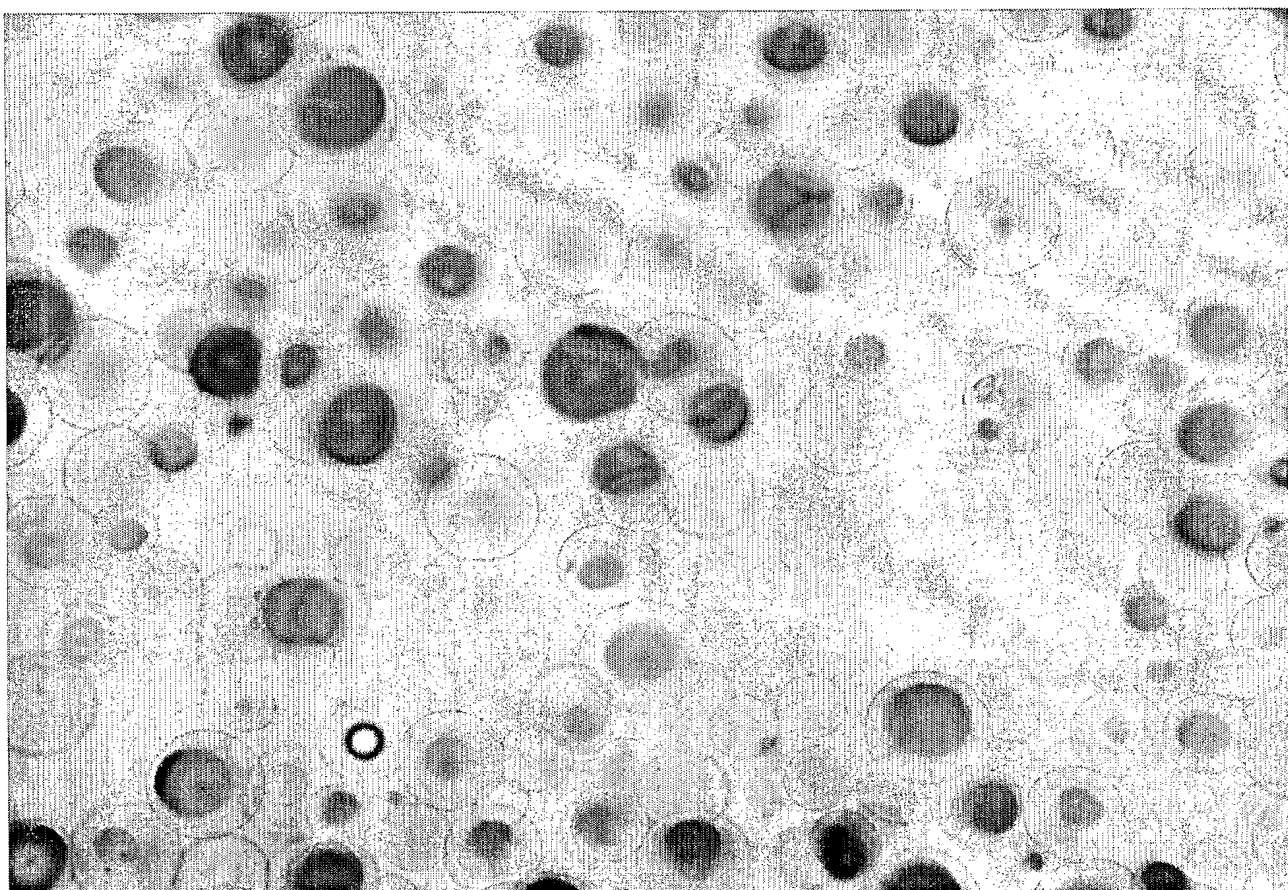
10 81. The method of claim 79, wherein the metal or metal compound is selected from the group consisting of phosphorus, yttrium and iodine.

82. The method of claim 80, wherein the metal or metal compound comprises gold.

15 83. The method of claim 82, wherein the gold is colloidal gold.

1/2

Figure 1



2/2

Figure 2



(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 May 2004 (21.05.2004)

PCT

(10) International Publication Number
WO 2004/040972 A3

(51) International Patent Classification⁷: **A61K 51/00**

(21) International Application Number:
PCT/US2003/033140

(22) International Filing Date: 17 October 2003 (17.10.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/423,547 4 November 2002 (04.11.2002) US

CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant: **BIOSPHERE MEDICAL** [US/US]; 1050 Hingham Street, Rockland, MA 02370-1052 (US).

(72) Inventor: **KROM, James, A.**; 41 Trowbridge Stret, Belmont, MA 02478 (US).

(74) Agents: **GORDON, Dana, M.** et al.; Patent Group, Foley Hoag LLP, 155 Seaport Boulevard, Boston, MA 02210-2600 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
14 October 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: RADIOISOTOPE-ASSOCIATED POLYMERIC HYDROGEL MICROSPHERES AND METHODS FOR PRODUCING AND USING THE SAME

(57) Abstract: The present invention relates to polymeric materials that are labeled with colloidal metals, preferably colloidal gold, to processes for producing the labeled polymeric material, and to methods of using the materials in prophylactic, therapeutic and cosmetic applications. Specifically, the invention relates to porous injectable and implantable microparticles, preferably microspheres, that are associated with radioactive colloidal metals such that the microparticles are visible or detectable under regular light, by radiological and/or magnetic resonance imaging techniques, or both. The microparticles having radioactive colloidal metals are particularly useful for embolization, radiation therapy, drug delivery, gene therapy, and other prophylactic or therapeutic medical applications.



WO 2004/040972 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/33140

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 51/00
US CL : 424/1.25, 1.29, 1.33

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.25, 1.29, 1.33, 1.21, 1.37; 600/1-8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,015,541 A (GREFF et al) 18 January 2000 (18.01.2000), see column 5, lines 42+ and columns 7 and 11.	1-83
X	US 6,248,057 B1 (MAVITY et al) 19 January 2001 (19.01.2001), see columns 5, 9 and 9-10.	1-83

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

24 February 2004 (24.02.2004)

Date of mailing of the international search report

05 AUG 2004

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Authorized officer

Michael G. Hartley

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

PCT/US03/33140

Continuation of B. FIELDS SEARCHED Item 3:

APS

search terms: hydrogels, hydrocolloids, microparticles, particles, microspheres, colloids, radiocolloids, radiotherapy, gold.